

Appl. No. 09/296,534
Amdt. Dated September 25, 2003
Reply to Notice of Non-Compliant Amendment of September 08, 2003

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method of determining the latent viral load in a host infected with HIV comprising,

contacting resting lymphoid mononuclear cells obtained from the host with an effective amount of an agent which activates an HIV virus integrated into the genome of the cells; and

detecting the expression of cell-surface gp120 on intact cells after the cells have been contacted with the agent and ~~determining the number of~~ counting the intact cells therein, wherein the number of intact cells expressing cell-surface gp120 is a measure of latent viral load.

Claim 2 (previously presented): The method of claim 1, comprising, prior to said contacting, obtaining the resting lymphoid mononuclear cells by the steps of:

- a) obtaining a sample cell population;
- b) depleting the sample cell population of cells expressing cell-surface gp120; and
- c) depleting sample cell population of cells expressing HLA-DR, whereby resting lymphoid mononuclear cells are obtained.

Claim 3 (previously presented): The method of claim 2, wherein the sample cells are depleted of gp120 expressing cells by the steps of:

- a) contacting sample cells with gp120-specific antibodies, said antibodies conjugated to a capture moiety, under conditions effective for the antibodies to attach to gp120 on the surface of cells, thereby forming labeled-cells;
- b) contacting the labeled-cells with capture moiety-specific antibody under conditions effective for the capture moiety-specific antibody to attach to the labeled-cells, thereby forming a complex-labeled cells; and
- c) removing the complex-labeled cells, thereby depleting sample cells of gp120+ cells.

Claim 4 (previously presented): The method of claim 3, wherein the capture moiety-specific antibody is conjugated to magnetic particles.

Claim 5 (previously presented): The method of claim 3, wherein the capture moiety is FITC and the capture moiety-specific antibody is FITC-specific antibody conjugated to a magnetic particles.

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Claim 6 (previously presented): The method of claim 4, wherein the magnetic particles are 10-100 nm in diameter.

Claim 7 (previously presented): The method of claim 5, wherein the magnetic particles are 10-100 nm in diameter.

Claim 8 (previously presented): The method of claim 3, wherein removing the complex-labeled cells is accomplished by a magnetic field acting on the magnetic particles.

Claim 9 (previously presented): The method of claim 2, further comprising:
separating CD4+ cells from the sample prior to said contacting.

Claim 10 (previously presented): The method of claim 2, further comprising:
separating CD8+ cells from the sample prior to said contacting.

Claim 11 (previously presented): The method of claim 2, wherein the depleting sample cell population of cells expressing HLA-DR is accomplished by flow cytometry cell sorting and said cells are labeled with a fluorochrome-labeled antibody specific for HLA-DR.

Claim 12 (previously presented): The method of claim 1, wherein the resting lymphoid mononuclear cells are obtained from a lymphoid tissue.

Claim 13 (previously presented): The method of claim 1, wherein the agent is phorbol ester or a cytokine.

Claim 14 (cancelled):

Claim 15 (previously presented): The method of claim 1, wherein the measure of latent viral load is compared to an established cell line harboring latent HIV-1.

Claim 16 (previously presented): The method of claim 15, wherein the cell line is OM-10.1, U1, or Jurkat cells.

Claim 17 (withdrawn): A method of treating a viral infection comprising
measuring the latent viral load in an HIV-infected patient; and
determining whether to administer to the patient an agent capable of activating an HIV virus integrated into the genome of a cell by the value of the latent viral load.

18. Claim 18 (currently amended): A method of determining latent viral load in a host infected with HIV comprising,
depleting a cell population obtained from the host of cells expressing cell-surface gp120 to obtain a depleted cell population, the original cell population comprising intact cells susceptible to HIV-infection ~~expressing cell-surface gp120~~, and
counting ~~determining~~, in said depleted cell population, the number of intact cells expressing cell-surface gp120, wherein said depleted cell population has been contacted

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with an agent which activates ~~capable of activating~~ HIV integrated into the genome of said cells under conditions effective for said agent to activate integrated HIV to obtain a determined a number of cells,

whereby said latent viral load in the host is the determined number of cells.

Claim 19 (currently amended): A method of determining latent viral load in a host infected with HIV comprising,

depleting a cell population obtained from the host of cells expressing cell-surface gp120 to obtain a depleted cell population, the original cell population comprising intact cells susceptible to HIV-infection ~~expressing cell-surface gp120~~,

contacting said depleted cell population with an agent which activates ~~capable of activating~~ HIV integrated into the genome of said cells under conditions effective for said agent to activate integrated HIV, and

counting determining, in said depleted cell population, the number of intact cells expressing cell-surface gp120 to obtain a determined a number of cells,

whereby said latent viral load in the host is the determined number of cells.

Claim 20 (new): The method of Claim 1, wherein the step of detecting the expression of cell-surface gp120 further comprises isolating the intact cells.

Claim 21 (new): The method of Claim 1, wherein the detecting the expression of cell-surface gp120 or the counting of intact cells is performed using flow cytometry or fluorescent microscopy.

Claim 22 (new): The method of Claim 20, wherein the detecting the expression of cell-surface gp120 or counting of intact cells is performed using flow cytometry or fluorescent microscopy.